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Iminosugars: Potential Inhibitors of Liver Glycogen Phosphorylase

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Abstract—The first synthesis of the single isomers (3*R*,4*R*,5*R*); (3*S*,4*S*,5*S*); (3*R*,4*R*,5*S*) and (3*S*,4*S*,5*R*) of 5-hydroxymethyl-piperidine-3,4-diol from Arecolin is reported, including the synthesis of a series of *N*-substituted derivatives of the (3*R*,4*R*,5*R*)-isomer (Isofagomine). The inhibitory effect of these isomers as well as of a series of *N*-substituted derivatives of the (3*R*,4*R*,5*R*)-isomer and selected hydroxypiperidine analogues on liver glycogen phosphorylase (GP) showed that the (3*R*,4*R*,5*R*) configuration was essential for obtaining an inhibitory effect at submicromolar concentration. The results also showed that all three hydroxy groups should be present and could not be substituted, nor were extra OH groups allowed if sub-micromolar inhibition should be obtained. Some inhibitory effect was retained for *N*-substituted derivatives of Isofagomine; however, *N*-substitution always resulted in a loss of activity compared to the parent compound, IC_{50} values ranging from 1 to 100 μM were obtained for simple alkyl, arylalkyl and benzoylmethyl substituents. Furthermore, we found that it was not enough to assure inhibitory effect to have the (*R,R,R*) configuration. Fagomine, the (2*R*,3*R*,4*R*)-2-hydroxymethylpiperidine-3,4-diol analogue, showed an IC_{50} value of 200 μM compared to 0.7 μM for Isofagomine. In addition, Isofagomine was able to prevent basal and glucagon stimulated glycogen degradation in cultured hepatocytes with IC_{50} values of 2–3μM.

Introduction

Iminosugars have attracted considerable interest due to their often effective and specific inhibition of various glycosidases involved in the intestinal degradation of carbohydrates.[1–3] However, for the treatment of diabetes this effect is weak and associated with side effects. It is generally recognised that the hepatic glucose output in type 2 diabetes is elevated and thus significantly contributes to hyperglycemia.[4–6] Glucose is produced by the liver from two different pathways: gluconeogeneis (de novo synthesis of glucose) and from glycogenolysis (breakdown of glycogen). However, it has been reported[7] that a substantial portion of the glucose produced by gluconeogenesis is cycled through the glycogen storage before efflux from the liver. This makes inhibition of the glycogenolysis a beneficial target to attack in the development of new anti-hyperglycemic agents. Since the discovery[8] of NN 42-1007 (Isofagomine) as a potent liver glycogen phosphorylase (GP) inhibitor, we have tested several other iminosugars for their ability to inhibit the GP enzyme (Fig. 1).

We here report on the SAR of a series of Isofagomine analogues together with a new synthetic approach to Isofagomine isomers starting from Arecolin.

Chemistry

Isofagomine has been prepared from D-lyxose in a multistep synthesis giving an overall yield of 11%,[1] and from 1,6 dianhydro-beta-D-glucopyranose yielding 10%.[9] Recently, a possible intermediate in the synthesis of Isofagomine isomers was prepared from a piperidone carboxylate derivative, and some isomers were prepared in a synthesis sequence similar to the one reported here.[10,11] An Isofagomine *N*-substituted with an extra glucose residue has been reported.[12]

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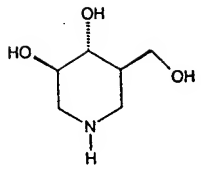


Isofagomine

NN 42-1007

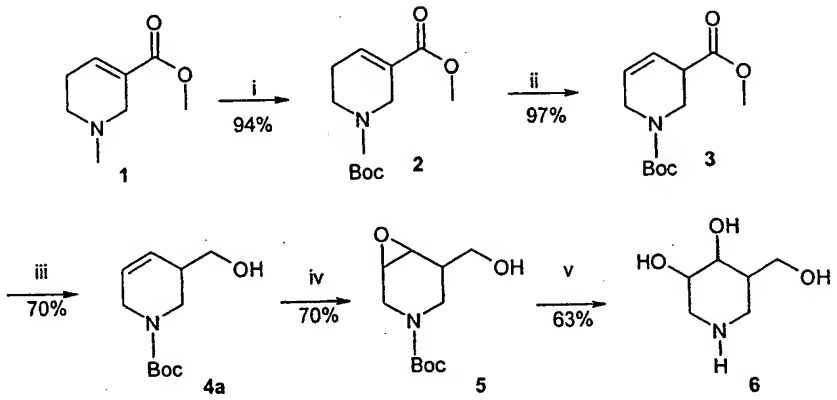
Figure 1.

In addition to the synthetic approach towards Isofagomine, we have also investigated a solid phase method for the preparation of *N*-alkylated derivatives of Isofagomine.

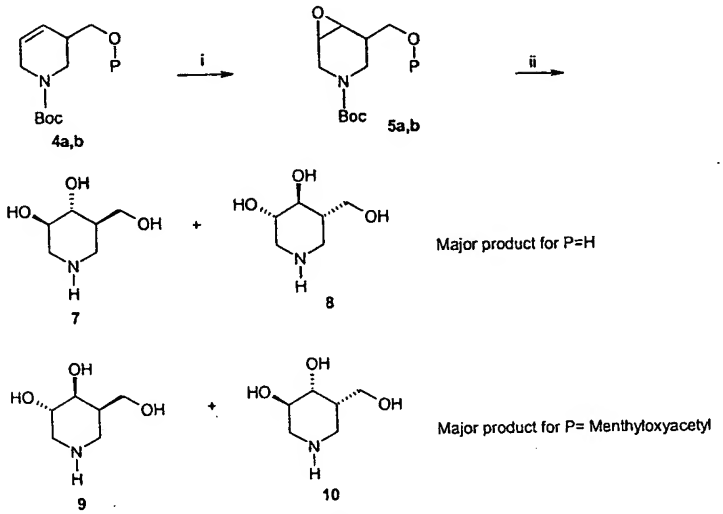
The reaction pathway from Arecolin to a mixture of Isofagomine isomers is depicted in Scheme 1. Arecolin **1** was demethylated by means of 1-chloroethyl chloroformate[13] followed by *N*-protection with Boc anhydride giving **2**.

Subsequent isomerisation using the method described by Allan and Fong[14] gave the isomerised product **3** in good yield. Reduction by means of sodium borohydride or lithium triethyl borohydride gave the unsaturated carbinol **4a** which was epoxidised by means of 3-chloroperbenzoic acid to give **5** as an isomer mixture, ring opening and deprotection proceeded by reflux in KOH solution (10%) to give the isomeric mixture **6**.

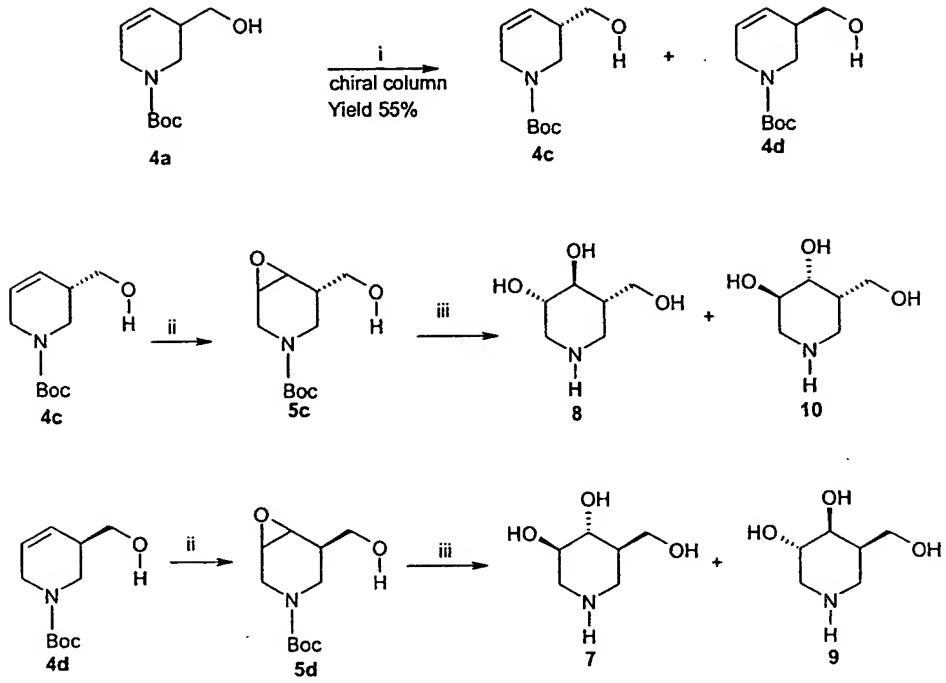
In order to prepare the single isomers of **6** we used two approaches; purification of the isomer mixture **6** on chiral plate followed by HPLC separation (Scheme 2), and



Scheme 1. Synthetic pathway from Arecolin to Isofagomine (compounds **4a**, **5** and **6** were isomer mixtures): (i), (a) ClCOOCHClMe, toluene; (b) MeOH; (c) $(Boc)_2O$, Et_3N, MeOH; (ii) LDA,THF, −78 °C; (iii) $LiEt_3BH$,THF; (iv) MCPBA, CH_2Cl_2, RT; (v) KOH (10%), reflux.



Scheme 2. Synthetic pathway to enantiomeric mixtures of Isofagomine: (a) P = H; (b) P = (−)-menthyloxyacetyl; (i) MCPBA, CH_2Cl_2, rt; (ii) (a) KOH (10%) reflux; (b) purification on chiral plate (Merchery Nagel), eluent Pr^iOH/25% NH_3 (3/1); (c) separation on HPLC, Merck LiChrosorb NH_2, eluent 70% CH_3 CN aq; identification by MS and NMR.



Scheme 3. Synthetic pathway to four stereoisomers of Isofagomine: (i) Chiracel OD or chiralpack AS, eluent Pr^iOH/heptane (1/99); (ii) MCPBA, CH_2Cl_2, rt; (iii) KOH (10%).

separation of the enantiomers **4a** by chiral HPLC followed by further reaction as described above (Scheme 3).

Separation of the isomer mixture of **6** as depicted in Scheme 2 was attempted with both an unprotected OH group and with a (−)-menthyloxyacetyl group, the latter in an attempt to achieve a greater difference in the chromatographic parameters. The epoxy compounds **5a** and **5b** were isolated as isomer mixtures and the subsequent step (ring opening and deprotection) resulted in a mixture of compounds **7**, **8**, **9**, and **10**. This mixture was subsequently separated on chiral plate followed by HPLC separation. The structure elucidation of the resulting enantiomer mixtures **7,8** and **9,10** showed that Isofagomine and its enantiomer (**7,8**) were the major products from the reaction sequence epoxidation/ring opening of **4a**, while the Isofagomine diastereomers (**9,10**) dominated from **4b**.

This difference is explainable from the directing effect of the hydroxyl with respect the menthyloxyacetyl group. No attempt was made to explore this directing effect in the epoxidation and ring opening step further. However, the NMR spectrum of **4a** indicated a preferred conformation in which one of the CH_2OH methylene protons strongly interacted with the hydrogens at the 2-methylene group.

The identity of the actual enantiomeric mixture formed was estimated by analysis of the NMR spectra, the **7,8** mixture being identical to Isofagomine, and the other mixture elucidated to be the Isofagomine diastereomers **9,10**.

In order to separate at an earlier step in the synthetic sequence, the unsaturated carbinol **4a** was separated in its enantiomers on a chiral column and the following reaction steps were performed on the pure enantiomers leading to diastereomeric mixtures which were separated by normal phase chromatography.

The yield in the separation step was found to 55%. No attempt was carried out to increase the yield. The enantiomer ratio was found to 1:3 the difference from 1:1 probably due to incomplete recovery, the ee of the enantiomers was >98%. Intermediates **5c** and **5d** were not isolated.

The product mixtures **7,9** and **8,10** were purified on a short silica-gel column using Pr^iOH/NH_3 aq as eluent, and the mixture was isolated as the hydrochloride salt for further separation by HPLC on a Merck LiChrosorb NH2 7 μm 25×250 mm column. **7** was collected between 6.75 and 7.5 min while **9** was eluted between 5 and 6 min. Their enantiomers **8** and **10** were collected after 6.25–7 and 5–5.75 min, respectively; the enantiomeric pair **7,8** thus being the isomer with the longest retention time.

Confirmation of relative stereochemistry of **7,8** and **9,10** was carried out by a NMR analysis.

Protons are denoted H-n (a or e) where *n* refers to the position labels. The methylene protons at C-2 and C-6 are tagged with an 'e' or an 'a' denoting equatorially-oriented and axially-oriented ^{1}H-nuclei, respectively.

The relative configuration of the two enantiomers, **7** and **8**, was confirmed by the observed vicinal ^{1}H-^{1}H-coupling constants ($^3J_{HH}$). Thus, large coupling constants attributable to trans-diaxial interaction between H-6a and H-5 ($J_{6a,5}$ = 12.0 Hz) and between H-3 and H-2a ($J_{3,2a}$ = 11.3 Hz) demonstrate that both H-3 and H-5 adapt axial positions. Furthermore, large coupling constants between H-4 and both its neighbours, H-5 ($J_{5,4}$ = 10.5 Hz) and H-3 ($J_{4,3}$ = 9.0 Hz), provide evidence for the *trans*-relation to both H-3 and H-5 in accordance with the depicted stereochemistry (**7** and **8**). The optical rotation of **7** was in accordance with the literature value.[9]

Similarly, the relative configuration of the enantiomeric pair, **9** and **10**, was confirmed by analysing the ^{1}H–^{1}H-coupling pattern. A large *trans*-diaxial coupling between H-6a and H-5 ($J_{6a,5}$ = 12.7 Hz) proved that H-5 is in the axial position, and a small coupling between H-5 and H-4 ($J_{5,4}$ = 2.9 Hz) places H-4 in the equatorial position. Finally, small coupling constants between H-3 and both H-2a and H-2e ($J_{3,2a}$ = 2.3 and $J_{3,2e}$ = 2.1 Hz) place H-3 equatorially, and conform with the expected stereochemistry (**9** and **10**).

The calculated ^{1}H data for the enantiomers were in accordance with the experimental values (data not shown).

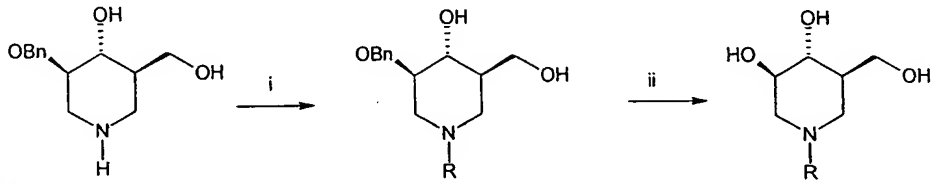
A series of *N*-alkylated Isofagomine derivatives were prepared by *N*-alkylation of 3-*O* benzylated Isofagomine[9] using standard *N*-alkylation methods like alkyl halogenid and base, or by reductive amination using aldehyde and cyanoborohydride (Scheme 4).

An alternative route was using the method described for Isofagomine[9] replacing ammonia with RNH_2.

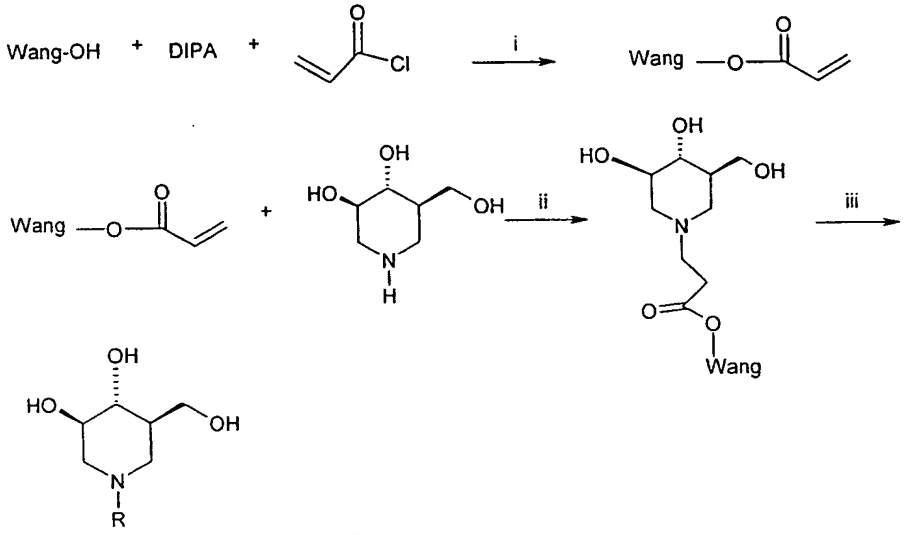
In order to prepare a large number of *N*-substituted compounds for a SAR investigation, we modified the solid phase method developed by Brown et al.[24] (Scheme 5).

Wang resin was reacted with vinyl chloroformate and subsequently with Isofagomine. The thereby formed solid phase bound Isofagomine was reacted with differently activated alkyl- or aryl-bromides, forming the quaternary ammonium compound. The desired *N*-substituted Isofagomine was liberated from the resin by treatment with DIPEA. Yields varied considerably (20–100%) and it was difficult to get rid of the DIPEA-salt and all the DMF at first, so HPLC purification was required in order to isolate the pure compounds. Proof of identity and yields were obtained form LC/MS and NMR measurements.

This method, although not optimised, opened for a variety of *N*-substituted derivatives for the SAR investigation. A representative selection of the approximately 100 compounds prepared is presented in Tables 1 and 2.



Scheme 4. Synthesis of *N*-substituted Isofagomine derivatives: (i) R-I, K_2CO_3, acetone or RCHO, $NaCNBH_3$, pH = 6, MeOH; (ii) Pd/C in EtOH/HCl (aq); Yields varying from 25 to 95%.



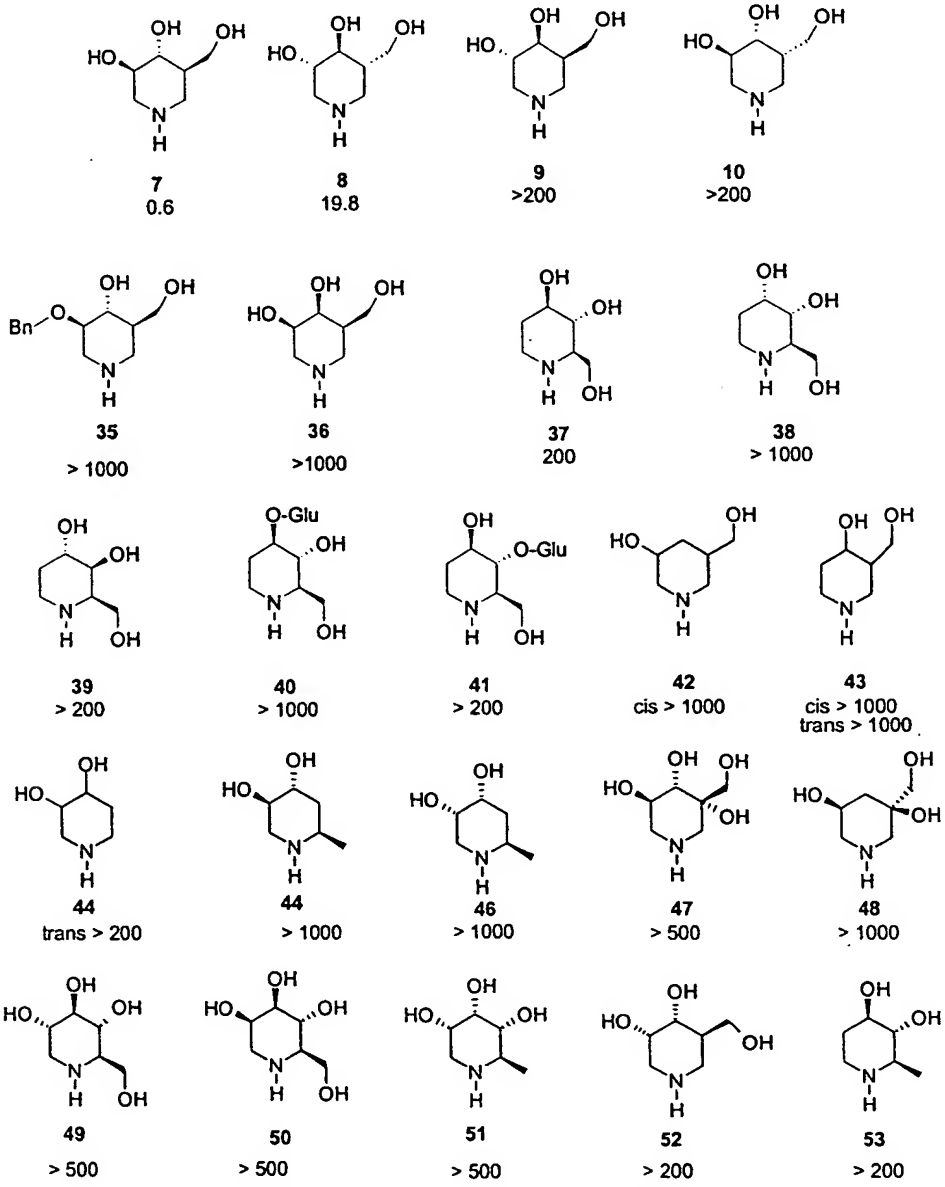
Scheme 5. Solid phase synthesis of *N*-substituted Isofagomine derivatives: (i) CH_2Cl_2, 7H, rt; (ii): DMF, 22 h, rt, wash; (iii) (a) R-Hal, DMF, 24 h, rt (b) DIPEA, DMF, 24 h, rt.

Table 1. Inhibition of pig liver glycogen phosphorylase (IC_{50} in μM) for *N*-substituted 7 (Isofagomine)

Compound no.	*N*-substituent	IC_{50} (μM)
7	-H	0.7
11	-Me	15
12	-Bu	20
13	-Allyl	4
14	-Propyn-3-yl	> 200
15	-1-Dodecyl	8
16	-Ac	60
17	$-CH_2CH_2COOH$	15
18	-Bn	46
19	$-CH_2CH_2Ph$	75
20	$-NO_2PhCH_2CH_2$	16
21	$-CH_2CH_2CH_2$-Ph	1
22	Cyclohexylprop-3-yl	2
23	$-CH_2CH{=}CHPh$	8

Table 2. Inhibition of pig liver glycogen phosphorylase for ring-substituted *N*-benzoylmethyl Isofagomine derivatives (IC_{50} in μM)

Compound no.	Phenyl substituent	IC_{50} (μM)
24	4-Br	40
25	4-H	7
26	3,4-diF	8
27	$3\text{-}CH_3$,4-Cl	27
28	4-Ph	70
29	$3\text{-}OCH_3$	8
30	$4\text{-}OCH_3$	10
31	$2,4\text{-di-}OCH_3$	> 100
32	$4\text{-}N(CH_2CH_3)_2$	> 100
33	$4\text{-}OCF_3$	80
34	3-F	6



Scheme 6. Inhibition of rat or pig liver glycogen phosphorylase (IC_{50} in μM) for selected Isofagomine analogues.

Table 3. IC_{50} values (nM) for inhibition of glycogen phosphorylase by Isofagomine[a]

	GP*a*	GP*b*
Rat liver	697±85 (4)	n.d.[b]
Pig liver	773±9 (5)	n.d.
Rabbit muscle	718±39 (3)	1215±180 (4)

[a]Results are means of the numbers in parentheses ±SEM. The Hill coefficients were calculated to be GP*a* rat liver: 1.10±0.05; GP*a* pig liver 1.07±0.03; GP*a* and GP*b* rabbit muscle: 1.07±0.14 and 1.13±0.06, respectively. No statistical significance among the groups was observed. [b]n.d. = not determined)

Biology

Data for inhibition of liver GP for a series of close analogues of Isofagomines including some compounds previously prepared or isolated from plant material are summarised in Scheme 6.

The data depicted in Scheme 6 show a surprising special structural requirements for inhibitory activity of this type of iminosugars as only compounds **7** and **8** showed inhibitory effect in the low μM range, and compound **7** was the only one giving an IC_{50} value below 1 μM.

Tables 1 and 2 show the inhibition data for *N*-substituted Isofagomine derivatives. These data show that some *N*-substitution is allowed. However, no *N*-substituted derivatives synthesised exhibit a better inhibitory effect on glycogen phosphorylase enzyme than Isofagomine.

Compounds **14**, **23**, **31**, and **32** prepared by parallel synthesis on Wang-resin were tested as 50% solutions, the other components being DIPEA salt and DMF as seen from the NMR spectra.

The test value was corrected for the concentration < 100%. However, the DIPEA salt/DMF mixture was shown not to inhibit the glycogen phosphorylase enzyme.

The inhibitory effect of the most potent compound Isofagomine (**7**) was furthermore characterised on GP obtained from various tissues (Table 3). Also, the ability of Isofagomine to inhibit glycogenolysis in cultured primary hepatocytes was investigated, with respect to inhibition of glucose production and the ability to retain glycogen levels. In hepatocytes, Isofagomine inhibited both basal and glucagon-induced glucose production dose-dependently with IC_{50} values of 3.0±0.4 and 2.0±0.5 μM, respectively (Fig. 2). In addition, 80% of the glycogen level could be retained at the highest dose of Isofagomine.

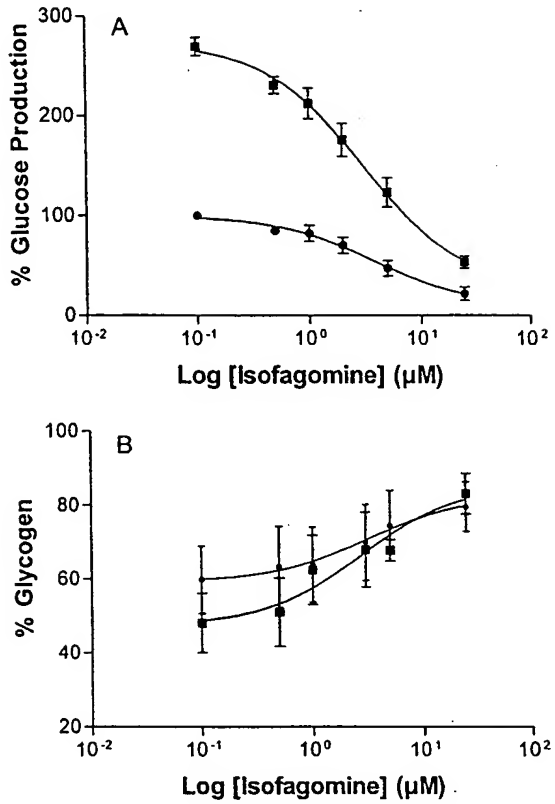


Figure 2. Effect of Isofagomine on basal and glucagon-stimulated glycogenolysis. (A) Basal (●) and glucagon-induced (0.5 nM glucagon) (■) glucose production were measured over 70 min (basal glucose production at 0 μM Isofagomine corresponds to 100%). Results are means±SEM (n = 4). (B) After 70 min of glycogenolysis the glycogen levels were determined: glycogen level after basal glycogenolysis (●) and glycogen levels after glucagon-induced glycogenolysis (0.5 nM glucagon) (■) (glycogen level before glycogenolysis corresponds to 100%). Results are means±SEM (n = 3).

Conclusion

The present investigation presents the first comparative data for inhibition of liver glycogen phosphorylase for six-membered iminosugars.

The synthetic methodology described gives the first synthesis of the pure enantiomer of Isofagomine (**8**) and add new data to the increasing set of literature reports on that matter. The structural requirements of the *N*-unsubstituted Isofagomine-like iminosugars for inhibition of liver glycogen phosphorylase clearly show that inhibitory effect in the low μM range can be achieved only when the compounds contain the *3R,4R,5R*- or the *3S,4S,5S*-configuration, as it appears from Scheme 6.

Furthermore, no extra OH group seems to be allowed (compounds **47**, **49** and **50**), nor is it allowed to substitute on the OH groups (**35**). It is surprising that neither Fagomine (**37**) nor Deoxynojirimycin (**49**) were found to be potent inhibitors of GP, bearing in mind that the five-ring analogue DAB was reported to show strong inhibition of glycogen phosphorylase[15,25] indicating that structures with the three hydroxy groups closer to the nitrogen are allowed, but obviously only when the ring size is diminished

It is furthermore clear that all three OH groups should be present, compounds **42**, **43 and 44** showing no activity. Substitution at other positions of the piperidine ring seems not to be allowed.

This strict requirement might indicate that the compounds bind in the catalytic site in the same manner as the natural ligand, with the three hydroxy groups mimicking the hydroxymethyl and the hydroxy groups in the 3 and 4 positions in a glucose moiety. Substitution on nitrogen always results in compounds with equal or less inhibitory effect, indicating that *N*-substitution does not destroy the interaction with the binding site.

The most potent compound inhibiting GP Isofagomine (7), was also able to inhibit glycogen breakdown using cultured hepatocytes as the functional model system.

Experimental

General procedures

Melting points (uncorrected) were measured on a Büchi 535 apparatus. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance DPX 200 MHz, or a 300 MHz apparatus, or a Bruker DRX400 or DRX600 instrument equipped with 5 mm selective inverse z-gradients probe heads operating at room temperature. Chemical shifts (δ-values) are reported relative to TMS. Coupling constants in Hz were directly measured from the 600 MHz ^{1}H NMR spectrum and confirmed by comparing experimental with computer simulated multiplet patterns (data not given).

Mass spectra were obtained on a Finnigan MAT TSQ 70 apparatus using a direct inlet system. The HPLC–MS analyses were performed on a PE Sciex API 100 LC/MS System using a Waters™ 3 mm×150 mm, 3.5 μ, C-18 Symmetry column and positive ion spray with a flow rate at 20 μL/min. The column was eluted with a linear gradient of 5–90% A, 85–0% B and 10% C for 15 min at a flow rate of 1 μL/min (solvent A = acetonitrile, solvent B = water and solvent C = 0.1% trifluoroacetic acid in water). Preparative HPLC was performed on Gilson HPLC equipped with 233XL combined injector/fraction collector, 305 and 306 pumps each with 25 μL/min pumping heads.

LC–MS analyses were obtained on a Hewlett Packard 1100 system, binary pump, degasser, injector, column oven and DAD detector and MSD single quadropole mass spectrometer. Microanalyses were performed by Novo Nordisk Analytical Department.

The syntheses of the following compounds were reported earlier: **37**, **38**, **39**, **40**, **41**, **50** and **53**[16–20]; **45**, **46**, **51**[21] and **36**.[22] Compounds **35**, **47**, **48** and **52** were received from M. Bols, Aarhus University; compound **49** is commercially available.

Methyl 1-*tert*-butoxycarbonyl-1,2,5,6-tetrahydropyridine-3-carboxylate (2). Arecoline (13.01 g) was dissolved in dry toluene (400 mL), 0 °C, in an inert atmosphere (N_2). α-Chloroethyl chloroformiate (14.00 g) was added and the mixture refluxed for 3 h and then evaporated. The residue was dissolved in MeOH (200 mL) and stirred at RT overnight. Evaporation gave a yellow oil which was stirred with 30 g K_2CO_3/300 mL H_2O for 10 min, extracted with DCM (3×100 mL), and dried over $MgSO_4$, evaporation gave crude methyl 1,2,5,6-tetrahydropyridine-3-carboxylate (95%).[23] Methyl 1,2,5,6-tetrahydropyridine-3-carboxylate (20.44 g, 145 mmol) and triethylamine (25 mL, 180 mmol) were dissolved in methanol (250 mL), and di-*tert*-butyl dicarbonate (65.48 g, 300 mmol) was added at rt. The mixture was heated at 60 °C for 30 min, evaporated to dryness and partitioned between water and ethyl acetate. The organic layer was isolated, dried over magnesium sulphate and evaporated to dryness in vacuo. Purification on silica-gel (eluent: DCM/methanol (19:1)) gave **2** as a yellow oil (yield: 34.9g, 94%). ^{13}C NMR ($CDCl_3$, ppm) δ 166.20, 155.14, 138.30, 128.5, 80.37, 52.07, 42.96, 28.81, 25.91. ^{1}H NMR ($CDCl_3$, ppm) δ 7.08 (1H, m), 4.10 (2H, m), 3.75 (3H, s), 3.48 (2H, t), 2.32 (2H, m) 1.48 (9H, s).

Methyl 1-*tert*-butylcarbonyl-1,2,3,6-tetrahydropyridine-3-carboxylate (3). Diisopropyl amine (7.64 mL) was dissolved in dry THF (50 mL), cooled to −78 °C in N_2 atmosphere followed by slow addition of BuLi (1.9 M, 30.50 mL). The temperature was allowed to rise to −10 °C for 30 min. Dry THF (50 mL) was added to the reaction mixture at −78 °C, then **2** (7.0 g) was added and the mixture stirred at −78 °C for 5 min. The reaction mixture was poured on a cooled NH_4Cl solution (1 M, 100 mL). The aqueous phase was adjusted to pH 10 and then extracted with Et_2O (3×100 mL). The organic phase was dried over $MgSO_4$, filtrated and evaporated, giving **3** (6.83 g, 97%). ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.86 (q, broad, 2H), 3.91 (q, broad, 2H), 3.72 (s, 3H), 3.52 (broad, 2H), 3.22 (broad,1H), 1.48 (s, 9H).

1-*tert*-Butoxycarbonyl-1,2,3,6-tetrahydropyridyl-3-methanol (4a). **3** (2.35 g, 9.8 mmol) was dissolved in dry diethyl ether (200 mL) under a nitrogen atmosphere. Lithium borohydride (0.6 g, 27.5 mmol) was slowly added followed by addition of 1.0 M lithium triethylborohydride in tetrahydrofuran (3 g, 28.3 mmol). The resulting mixture was refluxed for 1 h, poured into 1 M sodium hydroxide (100 mL) and extracted with methylene chloride. The organic layer was dried over magnesium sulphate, evaporated to dryness in vacuo and purified on a silica-gel column (eluent DCM/methanol (19/1)) to give **4a** as a colourless oil (Yield: 1.46 g, 70%) MS(EI): m/e 213 (M^+). ^{1}H NMR ($CDCl_3$, ppm) δ 5.76 (H4 and H5, b, 2H), 3.86 (H6, b, 2H), 3.61 (H2, dd, 1H), 3.50 (CH_2OH, dd, 1H), 3.38 (H2 and CH_2OH, b, 2H), 2.36 (H3, b, 1H), 1.48 (s, 9H). ^{13}C NMR ($CDCl_3$, ppm) δ 155.9 (CO), 126.7, 126.8 (C4 and C5), 80.1 ($C(Me)_3$), 63.3 (CH_2OH), 45.1 and 44.4 (C6), 44.4 and 43.2 (C2), 38.6 (C3), 27.7 (Me_3).

***tert*-Butyl 3-((1*R*,2*S*,5*R*)-2-isopropyl-5-methyl-1-cyclohexyloxy)acetoxymethyl-1,2,3,6-tetra-hydropyridine-1-carboxylate (4b).** (−)-Menthoxyacetyl chloride (1.03 g, 4.4 mmol) was slowly added to a solution of 1-*tert*-butoxycarbonyl-1,2,3,6-tetrahydropyridyl-3-methanol (**4a**) (0.59 g, 2.8 mmol) in DCM (50 mL) under a nitrogen atmosphere. The mixture was refluxed for 4 h, evaporated to dryness in vacuo and purified on a silica-gel column (eluent: methyl *tert*-butylether/methylene chloride

(1/20)) to give a diasteromeric mixture of **4b** as a colourless oil (yield: 0.54 g, 48%). A minor amount of the two isomers was separated on a chiral column (chiracel; eluent: Pr^iOH/heptane (5/95)) (Rt 9.6 and 11.2 min). MS: *m/e* 409 (M^+).^{13}C NMR ($CDCl_3$, ppm) δ 171.18, 155.34, 127.7 (m), 127.1 (m), 125.3 (m), 80.67, 80.59, 80.20, 66.29, 66.23, 65.33, 48.51, 43.4 (m), 42.9 (m), 40.35, 35.36, 34.81, 31.90, 28.82, 25.87, 23.68, 22.69, 21.38, 16.68.

1-*tert*-Butoxycarbonyl-1,2,3,6-tetrahydropyridyl-3-methanol (4c and 4d). **4a** (0.28 g crude) was separated in enantiomers on a chiracel OD (250–20 mm) column using 2-propanol/heptane (1/99) as eluent at a flow of 6 mL/min. **4c** (*S*) 1-*tert*-butoxycarbonyl-1,2,3,6-tetrahydropyridyl-3-methanol (40 mg) was collected from 16–23.5 min (ee >98%) and **4d** (*R*) 1-*tert*-butoxycarbonyl-1,2,3,6-tetrahydropyridyl-3-methanol (110 mg) was collected from 26.5–35.5 min (ee >98%). The NMR data were found in accordance with the ones reported for **4a**. The actual enantiomeric form was estimated from the data of the final products **4c** leading to **8** and **10**; **4d** leading to **7** and **9**.

(3*S*,4*S*,5*S*)-Hydroxymethyl-3,4-piperidinediol (8) and (3*R*,4*R*,5*S*)-hydroxymethyl-3,4-piperidinediol (10). MCPBA (230 mg, 70%) was dissolved in DCM (20 mL) and extracted with water (20 mL). The DCM phase was isolated and dried over $MgSO_4$. **4c** (48.5 mg) dissolved in DCM (10 mL) was added to the MCPBA solution, and the mixture was stirred at rt overnight. Subsequently, the reaction mixture was extracted with NaOH (10 mL), the water layer was extracted with DCM (3×10 mL), the combined DCM layers were pooled and evaporated. The residue was dissolved in KOH (10%), refluxed for 3 h and stirred at RT overnight. Evaporation gave a crystalline mass which was purified on a silica-gel column using 2-propanol/25% NH_3 as eluent. The crude product was dissolved in MeOH/H_2O, acidified with HCl (1 M) to pH 1 and evaporated to dryness. The resulting diastereomeric mixture was separated on a Merck LiChrosorb NH2. A 7 μm, 25×250 mm column. Flow 25 mL/min, 70% aqueous acetonitrile, 0.25 min/fraction. The samples were dissolved in 3 mL of mobile phase and injected. Every third fraction was analysed by FI–MS, EIC 148 *m/z* showed fraction containing 'relevant' molecular weight. The fractions containing the relevant compounds were all analysed by LC–MS. Flow 1 mL/min, 70%, acetonitrile, 30% 1 mM ammonium-acetate, column Merck LiChrosorb NH2 5 μm, 4×250 mm. **10** (4.7 mg) eluted between 5.0 and 5.75 min, and **8** (3 mg) eluted between 6.25 and 7 min. Both compounds eluted from the column as their HCl-salts. $[\alpha]_D$ for **8** was found to be −19° (MeOH,*c* = 0.20, 20 °C).[9]

(3*R*,4*R*,5*R*) 5-Hydroxymethyl-3,4-piperidinediol (7) and (3*S*,4*S*,5*R*) 5-hydroxymethyl-3,4-piperidinediol (9). **4d** (70 mg) was treated with MCPBA and KOH as described above for **4c**. The two diastereoisomers eluted as their HCl salts between 5 and 6 min (**9**) (8.2 mg), resp. 6.75 and 7.5 min (**7**) (2.4 mg). $[\alpha]_D$ for **7** was found to be +17° (MeOH,*c* = 0.16, 20 °C).[9] ^1H NMR (D_2O) **7** and **8** δ 2.73 (H-6a, $J_{6a,6e}$ = 12.7 Hz and $J_{6a,5}$ = 12.0 Hz), δ 3.34 (H-6e, $J_{6e,5}$ = 4.5 Hz and $J_{6e,2e}$ = 1.7 Hz), δ 1.85 (H-5, $J_{5,5'}$ = 3.4 Hz, $J_{5,5'''}$ = 6.4 Hz and $J_{5,4}$ = 10.5 Hz), δ 3.43 (H-4, $J_{4,3}$ = 9.0 Hz), δ 3.6 (H-3, J_{32a} = 11.3 Hz and $J_{3,2e}$ = 5.0 Hz), δ 2.68 (H-2a, $J_{2a,2e}$ = 12.5 Hz), δ 3.37 (H-2e), δ 3.82 (H-5′, $J_{5',5''}$ = 11.7 Hz), δ 3.70 (H-5″). ^1H NMR (D_2O) **9** and **10** δ 3.03 (H-6a, $J_{6a,6e}$ = 12.7 Hz and $J_{6a,5}$ = 12.7 Hz), δ 3.28 (H-6e, $J_{6e,5}$ = 4.0 Hz), δ 2.43 (H-5, $J_{5,5'}$ = 6.6 Hz, $J_{5,5''}$ = 7.0 Hz and $J_{5,4}$ = 2.9 Hz), δ 4.02 (H-4, $J_{4,3}$ = 3.9 Hz, $J_{4,2e}$ = 0.5 Hz), δ 4.07 (H-3, $J_{3,2a}$ = 2.1 Hz and $J_{3,2e}$ = 2.3 Hz), δ 3.36 (H-2a, $J_{2a,2e}$ = 13.7 Hz), δ 3.27 (H-2e), δ 3.73 (H-5′, $J_{5',5''}$ = 11.3 Hz), δ 3.63 (H-5″)

***tert*-Butyl 3-((1*R*,2*S*,5*R*) 2-isopropyl-5-methyl-1-cyclohexyloxy)acetoxymethyl-4,5-epoxypiperidine-1-carboxylate (5b).** A solution of 3-chloroperoxybenzoic acid (0.9 g, 5.2 mmol), *tert*-butyl 3-((1*R*,2*S*,5*R*)-2-isopropyl-5-methyl-1-cyclohexyloxy)acetoxymethyl-1,2,3,6-tetrahydropyridine-1-carboxylate (0.5 g, 1.2 mmol) and methylene chloride (150 mL) was stirred at rt for 20 h. An extra portion of 3-chloroperoxybenzoic acid (1.1 equiv) was added, and the mixture was refluxed for 2 h. The reaction mixture was extracted with 1 M sodium hydroxide and water, dried over magnesium sulphate and evaporated to dryness in vacuo. The residue was purified on a silica-gel column (eluent: heptane/ethyl acetate (5/1)) to give **5b** as an oil (yield: 0.34 g, 66%). ^{13}C NMR ($CDCl_3$, ppm) δ 173.16, 157.08, 82.71, 82.61, 68.28, 66.27, 65.78, 54.43, 53.9, 52.99, 52.35, 50.54, 44.5 (broad), 42.38, 41.22, 36,82, 33.92, 32.13, 30.77, 27.93, 25.72, 25.1, 23.4, 18.72.

***tert*-Butyl 3-hydroxymethyl-4,5-epoxypiperidine-1-carboxylate (5a).** was prepared from 1-*tert*-butoxycarbonyl-1,2,3,6-tetrahydropyridyl-3-methanol **4a** (40 mg) as described for **5b**. Isolated as an oil, yield 70%. LC–MS showed a 100% ELS purity, however, no molecular ion + 1 was detected due to split off of the Boc-group by the CF_3 COOH in the eluent. ^1H NMR ($CDCl_3$, ppm) 3.6–4.0 (m, 4H), 3.35 (t, 1H), 3.1–3.3 (m, 3H), 2.23 (m, 1H), 1.48 (s, 9H).

5-Hydroxymethyl-3,4-piperidinediol (9,10). **5b** (0.25 g, 0.59 mmol) and 10% aqueous potassium hydroxide were refluxed for 3 h. The reaction mixture was evaporated to dryness in vacuo and purified on a silica-gel column (Eluent: 2-propanol/25% NH_3 aq (3:1)) to give an oil (yield: 56 mg, 65%). Further purification on chiral TLC (Machery-Nagel 811058 plates; eluent: 2-propanol/25% NH_3 aq (3/1)) gave two isomers R_f 0.55 resp. 0.59. However they were not well separated and the mixture of the two isomers was subsequently separated on HPLC using a Merck Lichrosorb NH_2 column, eluent 70% CH_3CN aq. Only one fraction containing **9,10** was isolated and structure elucidated. The minor fraction was not further identified. ^1H NMR was as found for compound **9**.

MS: m/e 147 (M^+). ^{13}C NMR (D_2O, ppm) δ 71.52, 68.94, 65.64, 65.54, 60.59, 59.38, 47.03, 45.20, 44.57, 41.44, 40.98, 35.26.

5-Hydroxymethyl-3,4-piperidinediol (**7,8**) was prepared analogously from **5a** (0.65 g). Purification on chiral TLC (Machery-Nagel 811058 plates; eluent: 2-propanol/25% NH_3 aq (3/1)) gave two isomers R_f 0.52 resp. 0.58.

Subsequent separation on HPLC using a Merck Lichrosorb NH_2 column, eluent 70% CH_3CN aq. Only one fraction containing **7,8** was isolated and structure elucidated. The minor fraction was not further identified. ^1H NMR was as found for compound **7**.

Yield of **7,8**: 0.35 g (51%) as an oil, LC–MS M + 1 = 148.

1-Methyl-1,2,3,6-tetrahydro-3-pyridylmethanol (11). Diisopropyl amine (21.7 mL), was dissolved in dry THF (50 mL), cooled to −78 °C in N_2 atmosphere followed by addition of BuLi (2.2 M,70.80 mL). The temperature was allowed to rise to −10 °C for 30 min. Dry THF (150 mL) was added to the reaction mixture at −78°C, then Arecoline (12.61 g) was added and the mixture stirred at −78°C for 5 min. The reaction mixture was poured on a cooled saturated NH_4Cl solution (1 M, 300 mL). The aqueous phase was adjusted to pH 10 and then extracted with Et_2O (3×200 mL). The etheral layer dried over $MgSO_4$, filtrated and evaporated in vacuo, giving methyl 1-methyl-1,2,3,6-tetrahydro-pyridine-3-carboxylate (6.96 g, 55%).[14] Lithium triethylborohydride in tetrahydrofuran (1 M, 75 mL, 75 mmol) was slowly added to a solution of methyl 1-methyl-1,2,3-6-tetrahydropyridine-3-carboxylate (5 g, 31.8 mmol) in dry tetrahydrofuran (200 mL). The mixture was refluxed for 3 h, cooled to rt and poured into 1 M aqueous hydrochloric acid (150 mL). The aqueous layer was extracted with methylene chloride and pH was adjusted to 9 with aqueous sodium hydroxide. The solution was extracted with methylene chloride which was dried over magnesium sulfate and evaporated to dryness in vacuo. The residue was distilled (kugelrohr) (bp: 65–75 °C/0.28 mbar) to give **11** as an oil (yield: 1.9 g, 48%). ^{13}C NMR ($CDCl_3$, ppm) 127.28, 126.34, 66.94, 57.31, 54.83, 46.00, 37.58.

(3*R*,4*R*,5*R*)-1-Benzyl-3-benzyloxy-5-hydroxymethylpiperidin-4-ol (54). 4-*O*-Benzyl-2-deoxy-2-C-hydroxymethyl-D-glucopyranose[9] (6.0 g, 21.1 mmol) was dissolved in methanol (200 mL). Sodium periodate (21.4 g, 100 mmol) in water (200 mL) was added dropwise over 15 min. The mixture was stirred at 45–47 °C for 3.5 h. The precipitate was filtered off, and the mixture was concentrated and purified by flash chromatography on silica-gel using ethyl acetate as eluent. The purified product of 4-*O*-benzyl-2-deoxy-2-C-hydroxymethyl-D-xylo-pentodialdose was dissolved in methanol (50 mL) and benzyl amine (4.36 g, 40.7 mmol), and sodium cyanoborohydride (1.0 g, 15.9 mmol) was added. The mixture was stirred at rt for 3 days. Subsequently, the mixture was acidified to pH = 2 with concd hydrochloric acid and evaporated to dryness. The residue was dissolved in water (50 mL), pH was adjusted to 10 with aqueous sodium hydroxide, and the solution was extracted with methylene chloride. The organic phase was dried over magnesium sulphate, evaporated and purified on silica-gel using ethyl acetate as eluent. This afforded **54** as an oil (yield: 25%). ^1H NMR ($CDCl_3$, ppm) δ 1.6–1.85 (m, 3H), 2.8–3.5 (m, 7H), 3.7 (dd, 1H), 4.55 (dd, 2H), 4.8 (s, 2H), 7.1–7.3 (m. 10 H).^{13}C NMR ($CDCl_3$, ppm) δ 44.07, 55.02, 55.92, 61.65, 62.46, 72.43, 74.05, 80.20, 127.14, 127.33, 127.55, 127.89, 128.22, 128.27, 129.42, 137.48, 139.01, 172.84.

(3*R*,4*R*,5*R*)-3-Benzyloxy-5-hydroxymethyl-1-methylpiperidin-4-ol (51) was prepared analogous to reported for **54** from 4-*O*-benzyl-2-deoxy-2-C-hydroxymethyl-D-xylo-pentodialdose (2.78 g), using methyl amine hydrochloride (1.16 g) and sodium cyanoborohydride (0.62 g) in methanol (50 mL). Yield 1.15 g (38%). MS (M + 1 = 252. Elemental analysis: calculated for $C_{14}H_{22}NO_3Cl$, 2.9 H_2O: C, 49.45%; H, 7.71; N, 4.12. Found: C: 49.21%; H, 7.49; N, 4.38. ^1H NMR ($CDCl_3$, ppm) δ 1.8 (ddd, 2H), 1.9 (m, 1H), 2.3 (s, 1H), 2.75 (m, 1H), 3.1 (m, 1H), 3.2–3.8 (m, 6H), 4.6 (dd, 2H), 7.2–7.4 (m, 5H). ^{13}C NMR ($CDCl_3$, ppm) δ 42.07, 44.97, 55.59, 56.75, 63.11, 71.27, 75.06, 79.12, 126.94, 127.01, 127.65, 137.38.

(3*R*,4*R*,5*R*)-1-Butyl-5-hydroxymethyl-3,4-piperidinediol (12). (3*R*,4*R*,5*R*)-3-Benzyloxy-5-hydroxymethyl-4-piperidinol[9] (87 mg, 0.37 mmol), 1-iodobutane (81 mg, 0.44 mmol, 50 μL), potassium carbonate (154 mg, 1.1 mmol) and dry acetone (8 mL) were stirred for 24 h at 40 °C under a nitrogen atmosphere. The solvent was evaporated in vacuo, and the residue purified on a silica-gel column (eluent: ethyl acetate/methanol/25% ammonium hydroxide (6/1/1%)). This afforded the free base of (3*R*,4*R*,5*R*)-3-benzyloxy-1-butyl-5-hydroxymethyl-4-piperidinol (**56**) (yield: 72 mg, 67%). ^1H NMR (CD_3 OD, ppm) δ 7.4–7.2 (m, 5H), 4.70 (dd, 2H), 3.80 (dd, 1H), 3.54 (m, 1H), 3.34 (m, 1H), 3.25 (d, 1H), 3.08 (m, 2H), 2.38 (t, 2H), 1.85 (dd, 2H), 1.70 (m, 1H), 1.45 (m, 2H), 1.31 (m, 2H), 0.93 (t, 3H).

56 (105 mg, 0.4 mmol) was dissolved in ethanol (20 mL) containing aqueous hydrochloric acid (4 N, 0.3 mL), and palladium on carbon (10%, 30 mg) was added. The mixture was hydrogenated at 1 atm H_2-pressure for 3 h, filtered through Celite and concentrated in vacuo. Purification on a silica-gel column (eluent: ethyl acetate: methanol:25% ammonium hydroxide (4/1/1)) gave the free base of **12** (yield: 53 mg, 74%) as a semicrystalline compound. Mp 94–95 °C; MS(SP) m/e 203 (M^+). ^1H NMR (CD_3OD, ppm) δ 3.80 (dd, 1H), 3.51 (m, 2H), 3.06 (m, 3H), 2.43 (dd, 2H), 1.91 (dt, 2H), 1.75 (m, 1H), 1.53 (m, 2H), 1.33 (m, 2H), 0.92 (t, 3H).

In a similar way the following compounds were prepared.

((3*R*,4*R*,5*R*)-1-Dodecyl-5-hydroxymethyl-3,4-piperidinediol (15). (3*R*,4*R*,5*R*)-3-Benzyloxy-1-dodecyl-5-hydroxymethylpiperidin-4-ol (**57**) was prepared from (3*R*,4*R*, 5R)-3-benzyloxy-5-hydroxymethyl-4-piperidinol[9] and iododododecane. ^1H NMR (CD_3OD, ppm) 7.4–7.2 (m, 5H), 4.70 (dd, 2H), 3.80 (dd, 1H), 3.54 (m, 1H), 3.34 (m, 1H), 3.25 (d, 1H), 3.08 (m, 2H), 2.38 (t, 2H), 1.89 (dd, 2H), 1.78 (m, 1H), 1.5 (m, 2H), 1.3 (m, 18H), 0.9 (t, 3H). Reduction of **57** afforded **15**. Mp 59–60 °C; MS(SP) m/e 315 (M^+). ^1H NMR (CD_3OD, ppm) δ 3.80 (dd, 1H), 3.51 (m, 2H), 3.06 (m, 3H), 2.40 (dd, 2H), 1.91 (dt, 2H), 1.75 (m, 1H), 1.53 (m, 2H), 1.3 (m, 20H), 0.9 (t, 3H).

(3*R*,4*R*,5*R*)-5-Hydroxymethyl-1-(3-phenylpropyl)-piperidine-3,4-diol (21). From (3*R*,4*R*,5*R*)-3-benzyloxy-5-hydroxymethyl-1-(3-phenylpropyl)-piperidin-4-ol. ^1H NMR (CD_3OD, ppm) δ 7,2 (m, 5H), 3,80 (dd, 1H), 3.50 (m,

2H), 3.06 (m, 3H), 2.62 (t, 2H), 2.40 (m, 2H), 1.92–1.70 (m, 3H), 1.23 (m, 2H).

(3*R*,4*R*,5*R*)-1-(3-Cyclohexyl)propyl)-3,4-dihydroxy-5-hydroxymethylpiperidin (22). (3*R*,4*R*,5*R*)-3-Benzyloxy-5-hydroxymethyl-4-piperidinol[9] (0.397 g, 1.7 mmol) was dissolved in dry methanol (5 mL) and HCl in methanol (2 N) was added in excess. Evaporation to dryness in vacuo afforded the hydrochloride salt. The salt was dissolved in dry methanol (20 mL) and sodium cyanoborohydride (0.126 g, 2.0 mmol) was added. HCl in methanol (2 N) was added dropwise to obtain pH = 6 of the mixture (2 dr). 3-Cyclohexylpropionaldehyde (0.306 mL, 0.281 g, 2.0 mmol) was added, and the mixture was stirred under a nitrogen atmosphere for 18 h at rt. HCl in methanol (2 N) was added twice to maintain pH = 6 of the solution. Concentration in vacuo and purification of the residue on a silica-gel column (eluent: ethyl acetate: methanol/25% ammonium hydroxide (6/1/1)) afforded (3*R*,4*R*,5*R*)-3-benzyloxy-1-(3-(cyclohexyl)propyl)-5-hydroxymethylpiperidin-4-ol (yield: 0.58 g, 96%). ^{1}H NMR ($CDCl_3$, ppm) δ 7.4–7.2 (m, 5H), 4.62 (dd, 2H), 3.68 (m, 3H), 3.52–3.35 (m, 2H), 3.16 (m, 1H), 2.82 (m, 1H), 2.32 (t, 2H), 1,90 (m, 1H), 1.87–1.59 (m, 5H), 1.44 (m, 2H), 1.15 (m, 7H), 0.88 (m, 2H).

(3*R*,4*R*,5*R*)-3-Benzyloxy-1-(3-(cyclohexyl)propyl)-5-hydroxymethylpiperidin-4-ol (0.53 g, 1.48 mmol) was dissolved in ethanol (20 mL) containing aqueous hydrochloric acid (4 N, 0.3 mL) and palladium on carbon (10%, 50 mg) was added. The mixture was hydrogenated at 1 atm H_2-pressure for 3 h, filtred and concentrated in vacuo. Purification on a silica-gel column (eluent: ethylacetate/methanol/25% ammonium hydroxide (6/1/1)) gave the free base of **22** (yield: 0.21 g, 46%) as crystals. Mp 107–108 ° C. MS(FAB): *m/z* 272 (M + 1). ^{1}H NMR (CD_3OD, ppm) δ 3.80 (dd, 1H), 3.50 (m 2H), 3.06 (m, 3H), 2.37 (m, 2H), 1.88–1.58 (m, 8H),1.52 (m, 2H), 1.2 (m, 6H), 0.90 (m, 2H).

(3*R*,4*R*,5*R*)-5-Hydroxymethyl-1-(3-phenylallyl)-3,4-piperidinediol (23). To a suspension of Wang–OH resin (10 g, 9.2 mmol, Bachem, loading: 0.92 mmol/g) in methylene chloride *N,N*-diisopropylethylamine (15 mL, 87.8 mmol) and acryloyl chloride (10 mL, 122.7 mmol) were added. After stirring slowly for 7 h, the mixture was filtered and the resin washed with methylene chloride (500 mL) and methanol (500 mL). Drying in vacuo gave Wang resin–O–CO–CH=CH_2 (yield: 9.4 g). To a suspension of this resin (4 g, 3.7 mmol) in DMF (30 mL) a solution of 7 (0.9 g, 6.1 mmol) in DMF (5 mL) was added. The mixture was shaken for 22 h, filtered and the resin washed with DMF (4×25 mL), DCM (4×25 mL) and methanol (4×25 mL). The resin was dried in vacuo to give approx. 4.1 g material. 3-Bromo-1-phenyl-1-propene (180 mg, 0.91 mmol) was added to a suspension of this resin (190 mg, 0.17 mmol) in DMF (5 mL). The mixture was shaken for 24 h, filtered and the resin washed with DMF (5×5 mL), methylene chloride (3×5 mL) and DMF (2×5 mL). *N,N*-Diisopropylethyl amine (0.125 mL, 0.73 mmol) and DMF (3 mL) were added to the resin. The mixture was shaken for 24 h, filtered and the filtrate was concentrated to dryness in vacuo giving **23** as an oil (Yield: 35 mg, 78%). LC–MS m/e 264 (MH^+). ^{1}H NMR (CD_3OD, ppm) δ 7.5 (d, 2H), 7.3 (m, 3H), 6.9 (d, 1H), 6.35 (dt, 1H), 3.9 (d, 2H), 3.8 (dd, 1H), 3.7 (m, 2H), 3.55 (m, 2H), 3.4 (t, 1H), 2.76–3.0 (m, 2H), 1.95 (m, 1H).

The following compounds were prepared by use of the same solid support technique, the crude products were purified on HPLC, the identity confirmed by their LC–MS data and ^{1}H NMR spectra.

(3*R*,4*R*,5*R*)-1-Allyl-5-hydroxymethyl-3,4-piperidinediol (13). LC–MS m/e 188 (M + 1). ^{1}H NMR (CD_3OD, ppm) δ 5.9–6.1 (m, 1H), 5.5–5.7 (m, 2H), 3.3–3.9 (m, 8H), 2.7–3.0 (m, 2H), 1.95 (m, 1H). ^{13}C NMR (CD_3OD, ppm) 127.99, 126.77, 72.70, 70.14, 60.71, 60.41, 55.93, 43.12.

(3*R*,4*R*,5*R*)-1-Benzyl-5-hydroxymethyl-3,4-piperidinediol (18). LC–MS m/e 238 (M + 1). ^{1}H NMR (CD_3OD, ppm) δ 7.5 (m, 5H), 4.24 (s, 2H), 3.6–3.9 (m, 3H), 3.3–3.5 (m, 3H), 2.7–3.0 (m, 2H), 1.95 (m, 1H).

(3*R*,4*R*,5*R*)-1-(4-Bromophenacyl)-5-hydroxymethyl-3,4-piperidinediol (24). LC–MS 345 (M + 1).

(3*R*,4*R*,5*R*)-5-Hydroxymethyl-1-phenacyl-3,4-piperidinediol (25). LC–MS 266 (M + 1).

(3*R*,4*R*,5*R*)-1-(3,4-Difluorophenacyl)-5-hydroxymethyl-3,4-piperidinediol (26). LC–MS 302 (M + 1).

(3*R*,4*R*,5*R*)-5-Hydroxymethyl-1-(4-phenylphenacyl)-3,4-piperidinediol (28). LC–MS 342 (M + 1).

(3*R*,4*R*,5*R*)-5-Hydroxymethyl-1-(3-methoxyphenacyl)-3,4-piperidinediol (29). LC–MS 296 (M + 1).

(3*R*,4*R*,5*R*)-1-(4-Trifluoromethoxyphenacyl)-5-hydroxymethyl-3,4-piperidinediol (33). LC–MS 350 (M + 1).

(3*R*,4*R*,5*R*)-1-(3-Fluorophenacyl)-5-hydroxymethyl-3,4-piperidinediol (34). LC–MS 284 (M + 1).

(3*R*,4*R*,5*R*)-1-(4-Chloro-3-methylphenacyl)-5-hydroxymethyl-3,4-piperidinediol (27). LC–MS 314, 316 (M + 1). ^{1}H NMR (CD_3OD, ppm) δ 7.95 (d, 1H), 7.85 (dd, 1H), 7.6 (d, 1H), 5.0 (s, 2H), 2.9–3.9 (m, 8H), 2.5 (s, 3H), 2.1 (m, 1H).

(3*R*,4*R*,5*R*)-5-Hydroxymethyl-1-(2-(4′-methoxyacetophenone))-3,4-piperidinediol (30). LC–MS 296 (M + 1). ^{1}H NMR (CD_3OD, ppm) δ 8.0 (d, 2H), 7.07 (d, 2H), 4.7 (s, 2H), 3.9 (s, 3H), 2.7–3.85 (m, 8H), 2.05 (m, 1H).

(3*R*,4*R*,5*R*)-2,4-Dimethoxyphenacyl)-5-hydroxymethyl-3,4-piperidinediol (31). LC–MS 326 (M + 1).

(3*R*,4*R*,5*R*)-1-(4-Diethylaminophenacyl)-5-hydroxymethyl-3,4-piperidinediol (32). ^{1}H NMR (CD_3OD, ppm) δ 7.85 (d, 2H), 6.75 (d, 2H), 4.85 (s, 2H), 3.6–3.9 (m, 3H), 3.5 (q, 3H), 2.9–3.9 (m, 8H), 2.05 (m, 1H), 1.2 (t, 6H).

(3*R*,4*R*,5*R*)-5-Hydroxymethyl-1-phenethyl-3,4-piperidinediol (19). 7 (0.1 g), 2-phenyl-1-bromoethane (0.15 g), KI

(0.14 g) and K_2CO_3 (0.19 g) were mixed in DMF (20 mL) and stirred at rt for 10 days. NaOH (1 M, 50 mL) was added and the mixture extracted with DCM (2×50 mL), The aqueous phase was evaporated and purified twice on silica-gel (0.04–0.06 mm) using 2-propanol/25% NH_3 (3/1) as eluent. Yield 50 mg (29%) of an oil. LC–MS 252 (M + 1). HPLC (MeCN 65%, H_3PO_4 (pH = 3), flow 1 mL/min), rt 1.73 min, 98.5%.

(3*R*,4*R*,5*R*)-5-Hydroxymethyl-1-[2-(4-nitrophenyl)ethyl]-piperidine-3,4-diol (20). Preparation from **7** (0.1 g) and 2-(4-nitrophenyl)-1-bromoethane (0.19 g) as described for **19** gave 0.053 g (25%) of a yellow oil. Attempts to make the hydrochloride gave a hygroscopic mass which resulted in an oil; no further attempts were made to make crystals.

(3*R*,4*R*,5*R*)-1-Acetyl-5-hydroxymethyl-3,4-piperidinediol (16). (3*R*,4*R*,5*R*)-3-Benzyloxy-5-hydroxymethyl-4-piperidinol (0.1 g, 0.4 mmol) was dissolved in pyridine (0.5 mL) at 0 °C, and acetic anhydride (0.5 mL) was added dropwise over 1 min. Stirring at rt for 20 h and evaporation to dryness gave a crude product which was purified on silica-gel using ethanol as eluent (yield: 55%). The purified 3-benzyloxy-3-acetoxy-5-acetoxymethyl-1-acetylpiperidine (75 mg) was debenzylated in methanol at rt using 10% Pd/C as catalyst. Filtration and evaporation to dryness gave 50 mg of crude 4-acetoxy-5-acetoxymethyl-1-acetyl-3-piperidinol which was dissolved in dry methanol (4 mL) containing 50 µL of a 1% sodium methoxide in methanol. After stirring at rt for 4 days, the solution was evaporated to dryness to give **16** (yield: 35 mg). ^{1}H NMR (CD_3OD, ppm) δ 1.6 (m, 1H, H-5), 2.1 (s, 3H, O–Me), 2.4–2.7 (m, 1H, H-6_{ax}), 2.8–3.1 (m, 1H, H-4), 3.2–3.4 (m, 2H, H-2), 3.5–3.7 (m, 1H, H-5′), 3.7–4.1 (m, 2H, H-5′ + H-3), 4.4–4.6 (m, 1H, H-6_{equiv}). ^{13}C NMR (CD_3OD, ppm) δ 24.11, 24.16, 47.01, 48.17, 49.17, 50.14, 51.54, 51.78, 51.98, 54.81, 64.75, 64.84, 75.28, 75.88, 78.14, 78.36.

(3*R*,4*R*,5*R*)-3-(3,4-Dihydroxy-5-hydroxymethyl-piperidin-1-yl)propionic acid (17). To a suspension of Wang resin–O–CO–CH=CH_2 (2.0 g, 1.84 mmol) in DMF (15 mL) a solution of (3*R*,4*R*,5*R*)-5-hydroxymethyl-3,4-piperidinediol (0.8 g, 5.4 mmol) in DMF (3 mL) was added. The mixture was shaken for 22 h, filtered and the resin washed with DMF (2×20 mL), methanol (3×20 mL), DMF (2×20 mL), methylene chloride (2×20 mL) and methanol (2×20 mL). The resin was dried in vacuo to give approx. 2.2 g material. To a suspension of this resin (0.5 g, 0.46 mmol) in methylene chloride (5 mL) trifluoroacetic acid (5 mL) was added. The mixture was shaken for 20 min, filtered and the filtrate was evaporated to dryness *in* vacuo to give (3*R*,4*R*,5*R*)-3-(3,4-dihydroxy-5-hydroxymethyl-piperidin-1-yl)propionic acid as a yellow oil (yield: 40 mg, 40%). LC–MS m/e 220 (MH^+). ^{1}H NMR (CD_3OD, ppm) δ 3.3–3.9 (m, 8H), 2.8–3.1 (m, 4H), 1.95 (m, 1H).

***cis*-5-Hydroxymethyl-3-piperidinol (42).** Methyl 5-hydroxypyridine-3-carboxylate (5.0 g), acetic anhydride (4.95 mL), and sodium acetate (10 mg) were mixed in DCM (20 mL). After stirring at rt for 6 h H_2O (30 mL) was added, the organic phase was seperated, dried (Na_2SO_4) and evaporated yielding methyl 5-acetyloxypyridine-3-carboxylate (**58**) (6.63 g). The crude product was converted to the hydrochloride by use of HCl in MeOH (25%), the resulting salt was dissolved in MeOH (120 mL) and hydrogenated by use of PtO_2 (0.75 g) as catalyst. After shaking for 48 h the solution was filtered, evaporated and purified on a silica-gel column using MeOH/DCM (1/9) as eluent resulting in a rather low yield (1.5 g) of methyl 5-acetyloxypiperidine-3-carboxylate (**59**). This product was dissolved in THF (10 mL) and added dropwise to a solution of $LiAlH_4$ (0.33 g) in THF (10 mL). Due to difficulties in the isolation of the product, the crude product was acetylated by means of excess acetic anhydride/sodium acetate. The resulting product was purified on a silica-gel plate (MeOH/DCM (1/9) as eluent) yielding 0.15 mg of 1-acetyl-3-acetyloxymethyl-5-acetyloxypiperidine (**60**). This was *O*-deacetylated using MeONa (1% in MeoH) (0.15 mL) by stirring at rt overnight. Yield of 1-acetyl-5-hydroxymethyl-piperidine-3-ol (**61**) as an oil (0.35 g). MS m/e 173 (M+); ^{1}H NMR (MeOH) 4.42 (m, 1H), 3.82 (m, 1H), 3.5–3.0 (m, 4H), 2.68 (dd, 1H), 2.21 (dd, 1H), 2.01 (s, 3H), 1.87–2.09 (m, 1H), 1.6 (m, 1H). **61** (25 mg) was deacetylated by stirring with HCl (6 N, 1.5 mL) at 80 °C for 2 days, the reaction mixture was filtered and evaporated to dryness. Yield of **42** (10 mg, 40%). MS m/e 131 (M+). ^{13}C NMR 64.7, 64.5, 50.1, 46.8, 36.6, 36.1.

***cis*- and *trans*-3-Hydroxymethyl-4-piperidinol (43).** Ethyl 1-benzyl-4-oxopyridine3-carboxylate (15 g) was dissolved in dry MeOH (300 mL). $NaBH_4$ (30 g) was added to this solution over 20 min under N_2, the mixture was cooled during the addition, the cooling was removed whereby the temperature rose to 80 °C in 30 min. The mixture was further stirred at rt overnight. H_2O (10 mL) was added and the solution evaporated to dryness. EtAc (400 mL) was added and the mixture refluxed for 20 min, filtered hot and evaporated to dryness resulting in a crude oil (13.6 g). The mixture was purified on a silica-gel column (eluent: DCM/MeOH, 9/1). The first eluting compound was identified as the *trans* isomer of 1-benzyl-3-hydroxymethyl-4-piperidinol (**62**) (2.6 g). ^{13}C NMR (DMSO) 138.6, 128.7, 128.0, 126.8, 68.5, 62.1, 61.3, 55.0, 51.8–46.0, 38.4. ^{1}H NMR (DMSO) 7.2–7.35 (m, 5H), 4.55 (d, 1H), 3.62 (dd, 1H), 3.3–3.5 (b, 2H), 3.22 (d, b, 1H), 3.15 (m, 1H), 2.90 (d, b, 1H), 2.7 (m, 1H), 1.7 (m, 2H), 1.51 (m, 1H), 1.40 (m, 1H). The last eluted was identified as the *cis* isomer (2.4 g) of 1-benzyl-3-hydroxymethyl-4-piperidinol (**63**) ^{1}H NMR (DMSO) 4.35 (b, 1H), 3.78 (s, 1H), 3.4 (m, 3H), 3.31 (m, 1H), 2.5–2.3 (m, 3H), 1.68 (m, b, 1H), 1.58 (m, 2H). ^{13}C NMR (DMSO) 138.5, 128.8, 128.7, 126.8, 64.1, 62.4, 61.3, 51.0, 48.2, 43.3, 32.6. **62** and **63** (1.0 g) were debenzylated by means of Pd/C (10%) (0.25 g) catalysed hydrogenation at atm. pressure in abs. ethanol (100 mL) giving quantitative yield of crude product as oil.

***cis*-3-Hydroxymethyl-4-piperidinol.** ^{1}H NMR (MeOH) 4.05 (dd, 1H), 3.4–3.7 (m, 2H), 2.97 (m, 1H), 2.8–2.7 (m, 3H), 1.82–1.2 (m, 3H). ^{13}C NMR (MeOH) 66.6, 63.0, 44.4, 44.2, 41.5, 34.0.

***trans*-3-Hydroxymethyl-4-piperidinol.** ^{1}H NMR (MeOH) 3.60 (dd, 1H), 3.4–3.15 (m, 2H), 3.0–2.65 (m, 2H), 2.45–2.25 (m, 1H), 2.13 (t, 1H), 1.8–1.6 (m, b,1H), 1.45–1.12 (m, 2H). ^{13}C NMR (MeOH) 70.6, 62.9, 48.4, 48.1, 45.5, 36.1.

***trans*-3,4-Piperidinediol (44).** 1-Benzyl-1,2,3,6-tetrahydropyridine (4.87 g) was slowly added to a cooled mixture of acetic acid (31 mL) and H_2O_2 (7.5 mL, 30%). The resulting mixture was heated for 5 h at 80 °C. The solution was evaporated to 1/5 volume and Na_2CO_3 was added to pH 9. Satd NaCl (10 mL) was added and the mixture extracted with DCM×3. The organic phases were collected and evaporated to dryness. The resulting crystalline mass was dissolved in EtOH (25 mL) and acidified with HCl (6 M, 4.7 mL). The mixture was evaporated, redissolved in EtOH and precipitated with ether.

0.16 g of this crystalline mass was reduced by hydrogenation at atm pressure using Pd/C (10%) as catalyst in abs ethanol (20 mL), giving quantitative yield of **44.** Mp 128 °C (HCl–salt). MS 117 (M+). ^{13}C NMR (MeOH) 67.7, 67.5, 45.7, 41.2, 27.1. ^{1}H NMR (MeOH) 3.75 (m, 2H), 3.31 (dt, 2H), 3.3–2.92 (m, 2H), 2.2 (M, 1H), 1.8–1.6 (m, 1H).

Biology

Pig and rat liver GPa were semi-purified as described in Fosgerau et al.[25] Rabbit muscle GPa and GPb were obtained from Boehringer Manneheim. Enzyme activity was measured in the direction of glycogenolysis essentially as described by Bergmeyer.[26]

Rat hepatocytes were isolated and cultured as described by Andersen et al.[15] The effect of Isofagomine on glycogenolysis was measured in glucose-free medium in the absence or presence of glucagon (0.5 nM) as described previously.[15]

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